Long-term *in Vivo* Carcinogenicity Tests of Potassium Bromate, Sodium Hypochlorite, and Sodium Chlorite Conducted in Japan

by Yuji Kurokawa,* Shozo Takayama,† Yoichi Konishi,‡ Yoshio Hiasa,** Shogo Asahina,§ Michihito Takahashi,* Akihiko Maekawa,* and Yuzo Hayashi*

Long-term *in vivo* carcinogenicity tests of potassium bromate ($KBrO_3$), sodium hypochlorite ($NaClO$), and sodium chlorite ($NaClO_2$) have been conducted in Japan from 1977 to 1985. In these investigations, groups of approximately 50 male and 50 female F344 rats or B6C3F1 mice were given solutions of the compounds as their drinking water *ad libitum* at two dose levels determined on the basis of preliminary 13-week tests. Control animals were given distilled water.

The carcinogenic potential of $KBrO_3$ was tested by administering doses of 500 or 250 ppm to rats for 110 weeks. Significantly elevated incidences of renal cell tumors in males and females and mesotheliomas of the peritoneum in males as compared to controls were observed. When female mice were given $KBrO_3$ at doses of 1000 or 500 ppm for 78 weeks, no significant differences in tumor incidences between experimental and control groups were apparent.

$NaClO$ was administered to male and female rats, respectively, at doses of 1000 or 500 ppm and 2000 or 1000 ppm for 104 weeks. In mice, $NaClO$ was given at doses of 1000 or 500 ppm to either sex for 103 weeks. The incidences of tumors in $NaClO$-treated and control animals of both sexes were not significantly different in both rat and mouse studies.

$NaClO_2$ was given to rats of both sexes at a dose of 600 or 300 ppm for 85 weeks. No statistically significant differences were observed in the incidences of tumor formation between $NaClO_2$-treated and control groups of both sexes. $NaClO_2$ was administered to mice at a concentration of 500 or 250 ppm for 85 weeks. In males, the combined incidences of hyperplastic nodules and hepatocellular carcinomas of the liver in a low-dose group, and adenomas and adenocarcinomas of the lung in a high-dose group, were marginally increased compared to controls ($p < 0.05$). However, these incidences in treated males were within the range of values of historical control data in our program.

We concluded that $KBrO_3$ was carcinogenic in rats of both sexes. $NaClO$ was not carcinogenic in either rats and or mice under the conditions of the present studies. Although $NaClO_2$ was shown to be noncarcinogenic in rats, the results for mice were evaluated as inconclusive. Also the results of two-stage mouse skin carcinogenesis using $KBrO_3$, $NaClO$, and $NaClO_2$ are presented. The necessity for further testing of oxidant chemicals to determine potential carcinogenic and/or promoting effects is suggested in view of the recently proposed role of active oxygen species in carcinogenesis.

Introduction

The cooperative program in Japan for long-term assays of carcinogenicity was begun in 1974 with a subsidy for cancer research from the Ministry of Health and Welfare of Japan (*1,2*). The primary purpose of these

studies was not only to test the carcinogenic activity of environmental chemicals, but also to establish a correlation between the results in long- and short-term tests. The compounds were chosen for assessing carcinogenic potential based on their degree of human exposure, amount of production and use, strength of mutagenicity, previous data on carcinogenicity, etc. To date, 85 long-term *in vivo* tests using rats and/or mice have been conducted on 51 chemicals, including medical drugs, pesticides, and food additives (Y. Hayashi, unpublished data). In this paper, the results of the carcinogenicity bioassays of potassium bromate ($KBrO_3$), sodium hypochlorite ($NaClO$), and sodium chlorite ($NaClO_2$), which were performed in both rats and mice

*Department of Pathology, National Institute of Hygienic Sciences, 1-18-1, Kamiyooga, Setagayaku, Tokyo 158, Japan.

†National Cancer Center, 5-1-1, Tsukiji, Chuoku, Tokyo 104, Japan.

‡Department of Oncological Pathology, Cancer Center, Nara Medical College, 840, Shijomachi, Kashiharashi, Nara 634, Japan.

**Department of Pathology, Nara Medical College, 840 Shijomachi, Kashiharashi, Nara 634, Japan.

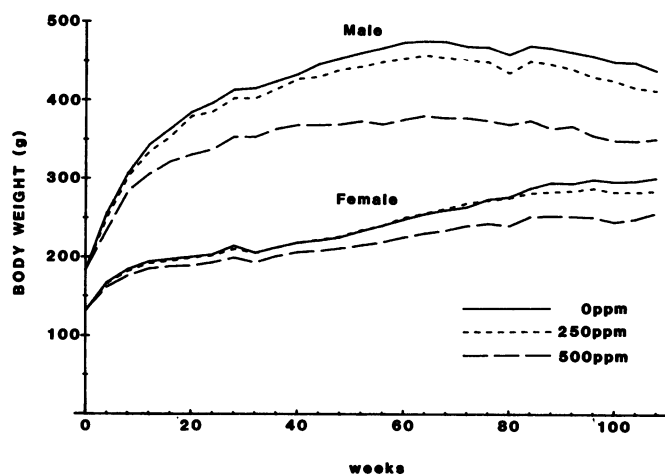§Department of Pathology, Fukushima Medical College, 5-75 Sugitsumacho, Fukushimashi, Fukushima 960, Japan.

FIGURE 1. Growth curves for male and female rats given KBrO₃.

as a part of this program from 1977 to 1985, are presented. Most of the carcinogenicity data described here are as yet unpublished because the tests have been completed only very recently.

Materials and Methods

Compounds and Sources

Potassium bromate, $KBrO_3$ (CAS.7758-01-2), is used as a bread- and flour-improving agent. It was acquired from Matsunaga Chemical Industry Ltd., Hiroshima, at a purity of 99.5%.
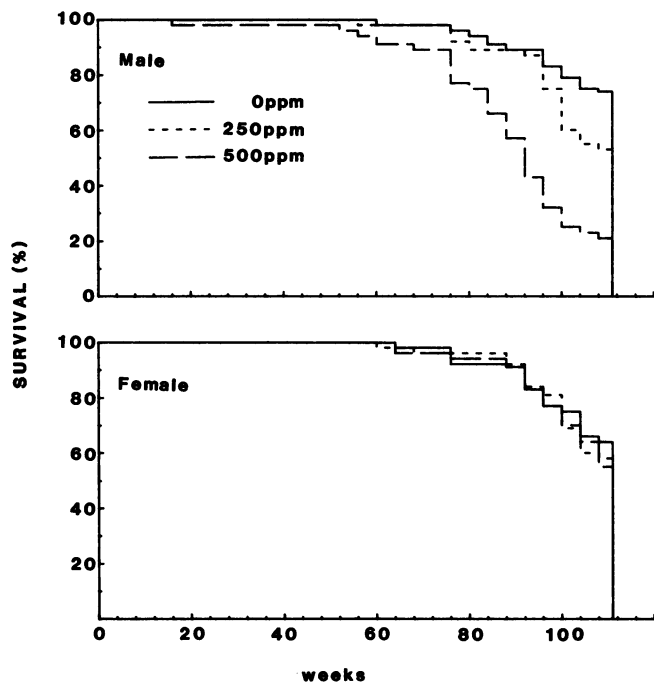

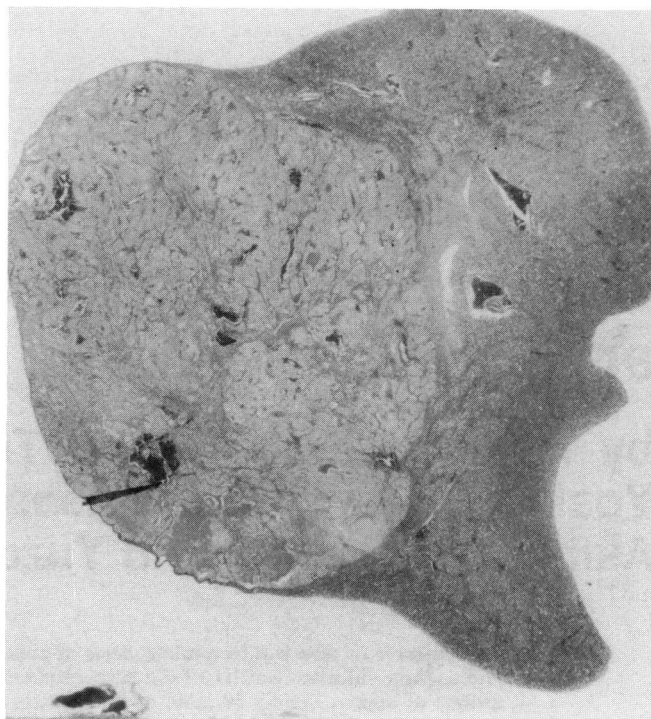FIGURE 2. Survival curves for male and female rats given KBrO₃.

FIGURE 3. Low power view of adenocarcinoma of the kidney in a rat given KBrO₃.

Sodium hypochlorite, NaClO (CAS.7681-52-9), is used as a disinfectant and bleaching agent. It was acquired from Thurumi Soda Co. Ltd., Tokyo. The effective concentration of chlorine was 14%.

Sodium chlorite, $NaClO_2$ (CAS.7758-19-2), is used as a bleaching agent. It was acquired from Wako Pure Chemical Industries Ltd., Osaka, at a purity of 99.4%.

Protocol for Carcinogenicity Testing

The experimental design of the long-term carcinogenicity tests was essentially similar to that published in the guidelines of the National Cancer Institute (3). Details are described in the bioassay of KBrO₃ in rats (4).

The test animals used were 4- to 6-week-old F344 rats or B6C3F1 mice (specific-pathogen-free) of both sexes, purchased from Charles River Japan Inc., Kanagawa. Groups of approximately 50 male and 50 female rats or mice were treated with the maximum tolerated dose (MTD) or ½ MTD, determined on the basis of 13-week toxicity tests. KBrO₃ and NaClO₂ were dissolved, and NaClO was diluted, in distilled water and administered orally to animals as their drinking water ad libitum. The doses chosen for the carcinogenicity tests were as follows: KBrO₃: 500 or 250 ppm for rats of both sexes and 1000 or 500 ppm for female mice. NaClO: 1000 or 500 ppm for male and 2000 or 1000 ppm for female rats and 1000 or 500 ppm for mice of both sexes. NaClO₂: 600 or 300 ppm for rats and 500 or 250 ppm for mice,
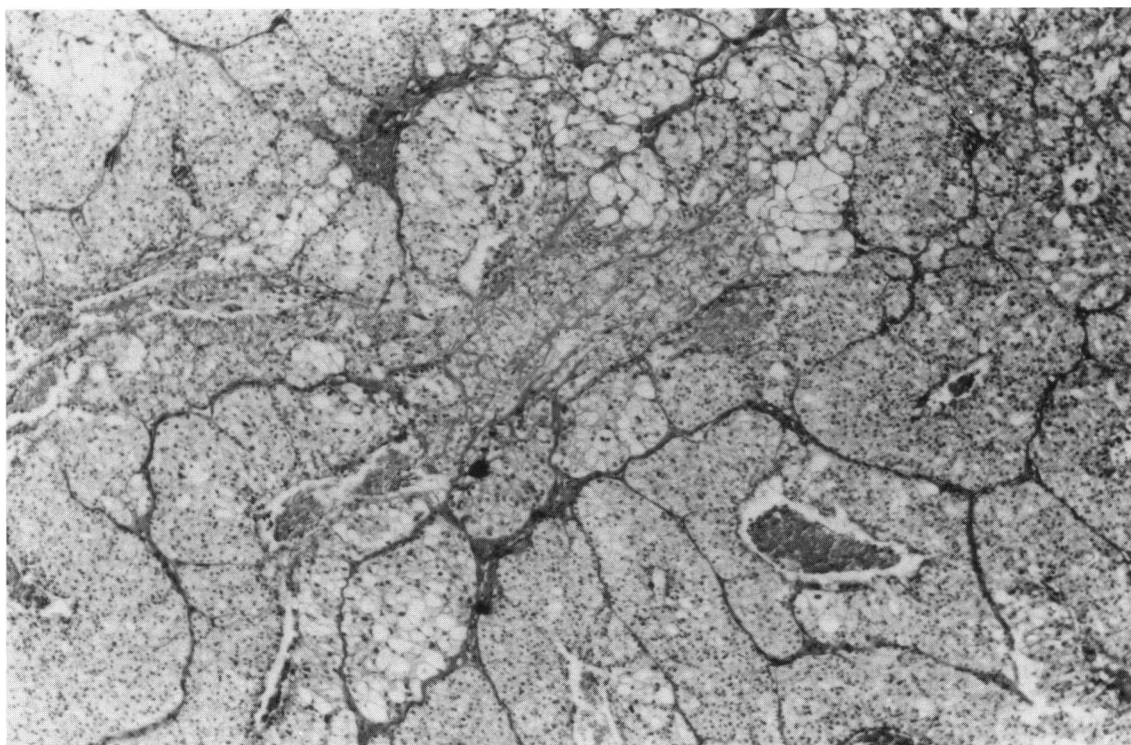
FIGURE 4. Detail of large adenocarcinoma of the kidney showing solid and tubular growth pattern. Hematoxylin and eosin (H&E) X110.

of both sexes. Control animals were given distilled water, and all animals were allowed free access to basal diet (CRF_1, Charles River). All three compounds were given continuously to rats and mice for at least 104 and 78 weeks, respectively, except in the case of the test for $NaClO_2$ in rats, which was terminated prematurely in week 85. The animals were observed daily. Dead animals and those found moribund were autopsied immediately. Consumption of water and body weights were recorded. At termination of the tests, all surviving animals were anesthetized by ether inhalation to collect blood for hematologic and serum biochemical analysis. At necropsy, organs were grossly examined, excised, and weighed. They were stained with hematoxylin and

eosin after routine histological processing. After detailed microscopic examination, the data were statistically analyzed using either the chi-square test, Fisher's exact probability test, or Student's t-test.

Results

Carcinogenicity Tests on KBrO₃

Rat. In males given 500 ppm of $KBrO_3$ (4), the dose was reduced to 400 ppm at week 60 because of a marked inhibition of body weight increase (Fig. 1). No apparent inhibition of body weight increase occurred in either sex given 250 ppm. The survival curves of male and female

Table 1. Incidence of renal cell tumors in rats given KBrO₃.

Sex	Group KBrO₃ dose, ppm	Group Effective no. of rats[a]	Mean induction time ± SD, weeks	Earliest renal cell tumor, weeks	No. (%) of rats bearing Renal cell tumors	No. (%) of rats bearing Adeno-carcinomas	No. (%) of rats bearing Adenomas
Male	500	52	88.9 ± 18.9	14	46(88)*	44(85)*	5(10)†
	250	53	103.7 ± 9.1	77	32(60)*	24(45)*	10(19)†
	0	53	111.0 ± 0	111	3 (6)	3 (6)	0 (0)
Female	500	49	107.9 ± 5.6	85	39(80)*	36(69)*	9(17)†
	250	50	107.6 ± 5.8	89	28(56)*	21(40)*	8(15)†
	0	47	—	—	0 (0)	0 (0)	0 (0)

[a]Males and females surviving longer than 14 and 85 weeks, respectively, when the first renal cell tumors were found.
*$p<0.001$.
†$p<0.01$

Table 2. Incidence of mesotheliomas in male rats given KBrO₃.

| KBrO₃ dose, ppm	Effective no. of rats[a]	Mean induction time ± SD, weeks	Earliest mesothelioma found, weeks	No. (%) of rats with mesotheliomas
500	46	91.0 ± 10.7	73	28(59)*
250	52	95.0 ± 14.4	72	17(33)†
0	53	98.8 ± 12.1	80	6(11)

[a]Males surviving longer than 72 weeks when the first mesothelioma was found.
*$p<0.001$.
†$p<0.05$.

rats given KBrO₃ for 110 weeks are shown in Figure 2. Dead or moribund rats appeared earlier among males given 500 ppm. For females, the survival curves of treated and control groups were very similar. All surviving animals were sacrificed in week 111. No significant differences in water intake were apparent between treated and control groups. Daily intakes of KBrO₃ (mg/kg body weight/day) were, in males, 27.7 and 12.5 and, in females, 25.5 and 12.5, in high- and low-dose groups, respectively.

For detailed histopathologic examination for kidney, 10–15 step-serial sections were made per kidney in this study. As a result, high incidences of renal cell tumors
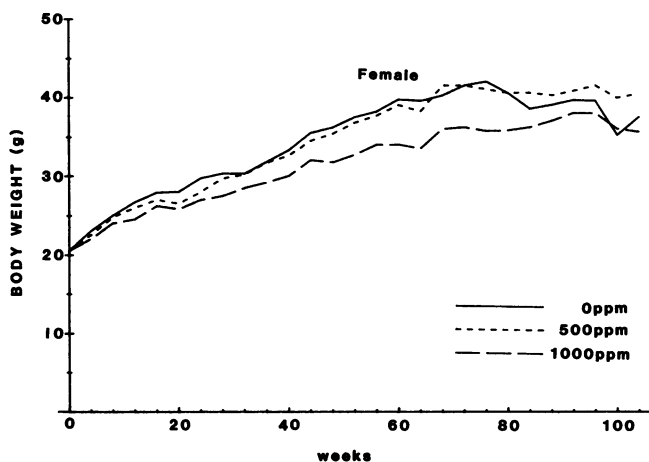
FIGURE 5. Growth curve for female mice given KBrO₃.
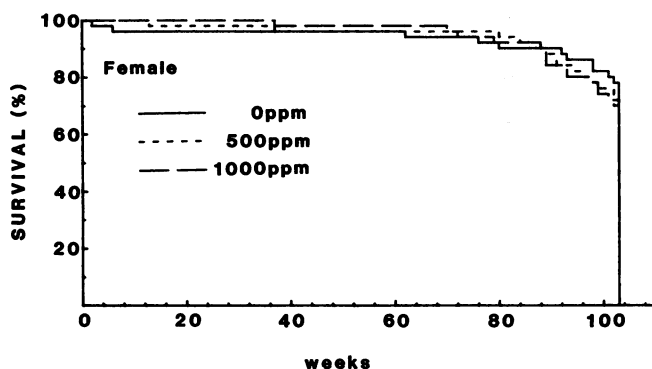
FIGURE 6. Survival curve for female mice given KBrO₃.
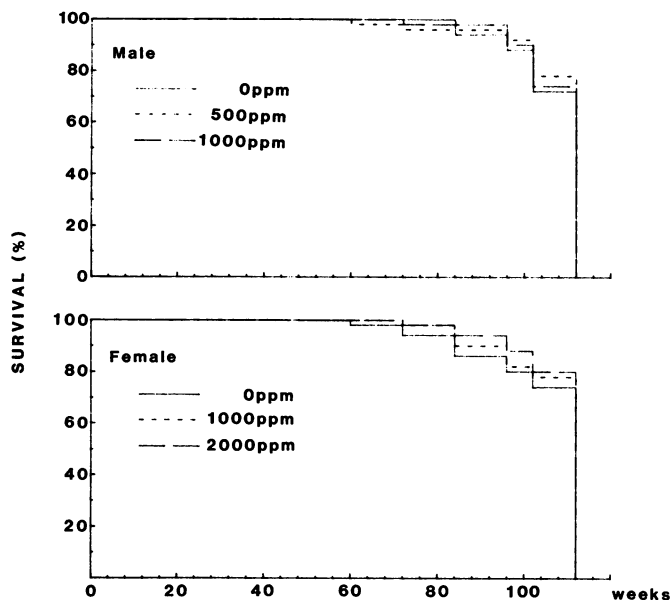
FIGURE 7. Survival curves for male and female rats given NaClO.

in KBrO₃-treated rats of both sexes were noted. Most renal cell tumors were microscopic in size, although some were visible as round yellowish-white or grayish projections from the renal cortex (Fig. 3). Histologically, renal cell tumors were classified as adenocarcinomas and adenomas, the former appearing irregularly contoured as if several small nodules were aggregated. They were mostly localized in the cortical areas and exhibited solid growth patterns, although in some cases trabecular, tubular, or papillary patterns were also observed (Fig. 4). Apparent infiltrative growth was seen in cases with grossly large tumors. The tumor cells were found to have either clear cytoplasm (clear cell), eosinophilic granular cytoplasm (granular cell), or homogenously basophilic cytoplasm (dark cell). Adenomas appeared as oval, solitary nodules consisting of closely packed polygonal cells. They were well circumscribed with thin fibrous capsules. Besides these distinct neoplastic lesions, dysplastic foci, considered preneoplastic changes, were frequently observed. Table 1 shows the incidences of adenocarcinomas and adenomas and their combined incidences as renal cell tumors. Renal cell tumors developed in 46 (88%), 32 (60%), and 3 (6%) of the males and 39 (80%), 28 (56%), and 0 (0%) of the females given 500, 250, and 0 ppm of KBrO₃, respectively. The percentages of animals developing renal cell tumors among animals treated with KBrO₃ were 74.3% (78/105) males, 67.7% (67/99) females, and 71.1% (145/200) all animals. Incidences of renal cell tumors in treated groups of both sexes were significantly different from those in control groups ($p<.001$). Also, the incidences of adenocarcinomas and adenomas of the kidney in both sexes were significantly different at levels of $p<0.001$ and $p<0.01$, respectively.

In addition, high incidences of tumors of the peritoneum, all diagnosed as mesotheliomas, were observed

Table 3. Histopathologic diagnoses of tumors occurring at relatively high incidence in rats given $KBrO_3$.

	Male			Female		
	500 ppm $KBrO_3$	250 ppm $KBrO_3$	0 ppm	500 ppm $KBrO_3$	250 ppm $KBrO_3$	0 ppm
Effective no. of rats (examined histologically)	52	53	53	52	52	52
No. (%) of tumor-bearing rats	52 (100)	53 (100)	53 (100)	43 (83)	48 (92)	44 (85)
Pituitary	3 (6)	8 (15)	12 (23)	12 (23)	15 (29)	21 (40)
Adenoma (chromophobic)	3	8	12	12	15	21
Ear duct	3 (6)	—	—	—	1 (2)	1 (2)
Squamous cell carcinoma	3	—	—	—	1	1
Mammary gland	3 (6)	7 (13)	14 (26)	2 (4)	6 (12)	3 (6)
Fibroma	2	4	4	1	—	1
Fibroadenoma	—	—	7	—	3	2
Adenoma	—	3	3	—	3	—
Adenocarcinoma	1	—	—	1	—	—
Thyroid	15 (29)	14 (26)	10 (21)	12 (23)	10 (19)	3 (6)
C-cell adenoma	2	5	7	4	6	2
C-cell adenocarcinoma	—	—	—	—	1	1
Follicular adenoma	2	—	—	1	1	—
Follicular adenocarcinoma	5	2	2	2	—	—
Papillary adenoma	1	2	—	—	—	—
Papillary adenocarcinoma	5	5	1	5	2	—
Pancreas	1 (2)	2 (4)	7 (13)	1 (2)	—	3 (6)
Acinar cell adenoma	—	—	1	—	—	—
Insuloma	1	2	4	1	—	2
Malignant insuloma	—	—	2	—	—	1
Adrenal	9 (17)	7 (13)	6 (12)	1 (2)	2 (4)	9 (17)
Pheochromocytoma	8	7	6	1	1	4
Malignant pheochromocytoma	1	—	—	—	—	—
Cortical adenoma	—	—	—	—	1	3
Ganglioneuroma	—	—	—	—	—	1
Ganglioneuroblastoma	—	—	—	—	—	1
Lung	6 (12)	5 (10)	5 (10)	3 (6)	4 (8)	—
Adenoma	6	4	4	2	4	—
Adenocarcinoma	—	1	1	1	—	—
Large intestine	3 (6)	2 (4)	—	3 (6)	3 (6)	1 (2)
Adenoma	3	—	—	1	2	—
Adenocarcinoma	—	2	—	2	1	—
Fibrosarcoma	—	—	—	—	—	1
Liver	6 (11)	7 (13)	2 (4)	2 (4)	1 (2)	2 (4)
Neoplastic nodule	5	5	2	2	1	2
Hepatocellular carcinoma	1	1	—	—	—	—
Cholangioma	—	1	—	—	—	—
Testis	52 (100)	50 (94)	52 (98)	—	—	—
Interstitial cell tumor	52	50	52	—	—	—
Uterus	—	—	—	4 (8)	4 (8)	10 (19)
Endometrial stromal polyp	—	—	—	2	3	9
Adenoma	—	—	—	1	1	1
Fibrosarcoma	—	—	—	1	—	1
Spleen	11 (21)	12 (23)	9 (17)	12 (23)	14 (27)	13 (25)
Leukemia (mononuclear cell)	11	121	9	11	14	13
Malignant hemangioendothelioma	—	—	—	1	—	—
Skin	5 (8)	1 (2)	2 (4)	—	1 (2)	—
Squamous cell papilloma	—	—	1	—	—	—
Squamous cell carcinoma	1	—	—	—	1	—
Preputial gland/clitoral gland	2 (4)	5 (9)	3 (6)	3 (6)	4 (8)	2 (4)
Adenoma	2	5	2	3	4	2
Squamous cell carcinoma	—	—	1	—	—	—

in males treated with $KBrO_3$ (Table 2). The incidences of mesotheliomas in male rats given 500 or 250 ppm were significantly different from that in controls, respectively, at $p<0.001$ and $p<0.05$ levels.

Table 3 summarizes the histopathologic diagnosis of tumors occurring at relatively high incidences in various organs other than in the kidney and peritoneum. The incidences of tumor-bearing animals were very high in both control and treated groups of both sexes. This phenomenon resulted from the high incidences of tumors of kidney, testis, peritoneum, thyroid, pituitary, mammary gland, and spleen.

Nonneoplastic renal tubular lesions occurring in $KBrO_3$-treated rats included various degenerative, necrotic, and regenerative changes. Hyaline cast in the tubular lumen, and hyaline droplets, eosinophilic bodies, and brown pigments in the tubular epithelium were also commonly observed. Biochemical analysis of the
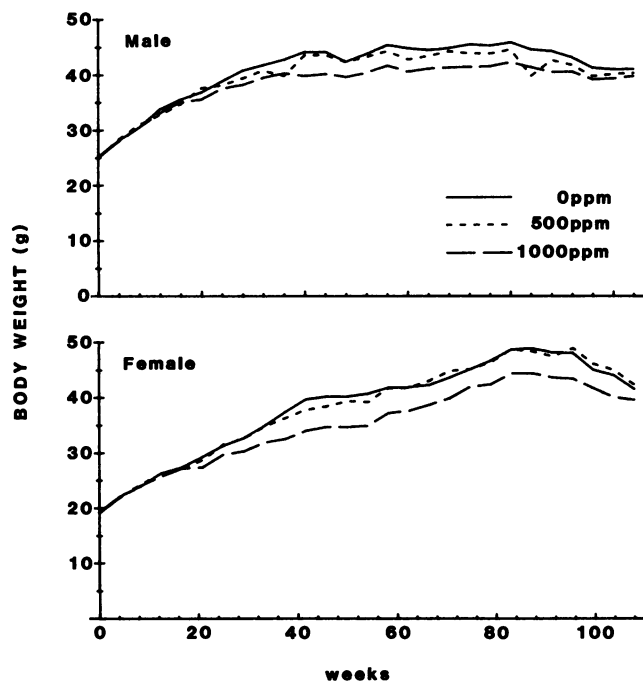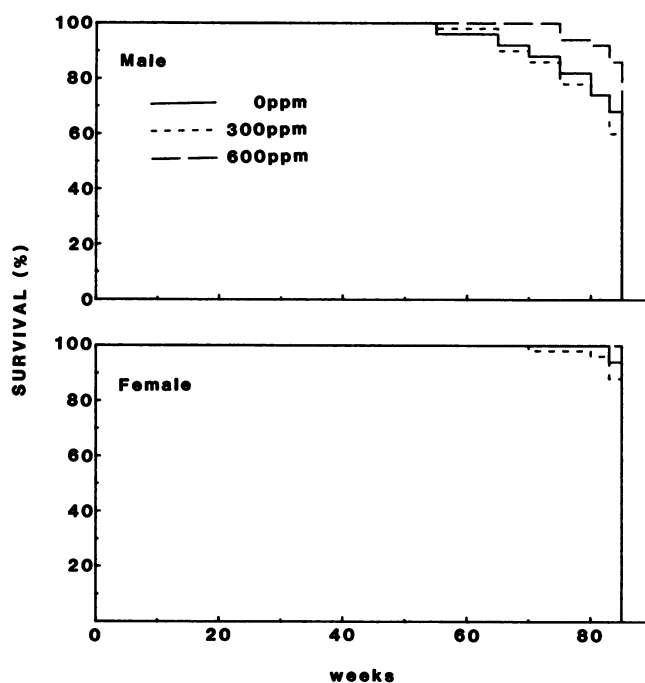
Table 4. Histopathologic diagnoses of tumors occurring in female mice given KBrO₃.

	Female		
	1000 ppm KBrO₃	500 ppm KBrO₃	0 ppm
Effective no. of mice	47	48	46
No. (%) of tumor-bearing mice	22 (47)	16 (33)	15 (33)
Pituitary	1 (2)	—	3 (7)
Adenoma	1	—	3
Harderian gland	—	2 (4)	—
Adenoma	—	2	—
Mammary gland	1 (2)	1 (2)	—
Adenocarcinoma	1	1	—
Lung	8 (17)	3 (6)	3 (7)
Adenoma	5	1	2
Adenocarcinoma	1	2	1
Squamous cell carcinoma	2	—	—
Forestomach	2 (4)	—	—
Squamous cell carcinoma	2	—	—
Duodenum	—	—	1 (2)
Epithelial polyp	—	—	1
Liver	6 (13)	3 (6)	3 (7)
Adenoma	3	3	—
Hemangioma	1	—	—
Carcinoma	2	—	3
Pancreas	1 (2)	—	—
Adenoma	1	—	—
Spleen	3 (6)	1 (2)	—
Hemangioma	1	1	—
Hemangiosarcoma	2	—	—
Lymph node	7 (15)	4 (8)	4 (9)
Lymphoma	7	4	4
Kidney	2 (4)	—	1 (2)
Adenoma	1	—	1
Adenocarcinoma	1	—	—
Adrenal	—	—	1 (2)
Adrenocortical tumor	—	—	1
Uterus	—	3 (6)	1 (2)
Squamous polyp	—	1	1
Leiomyoma	—	1	—
Endometrial sarcoma	—	1	—
Abdominal cavity	—	1 (2)	—
Fibroma	—	1	—
Skin	1 (2)	1 (2)	1 (2)
Fibrosarcoma	1	1	1
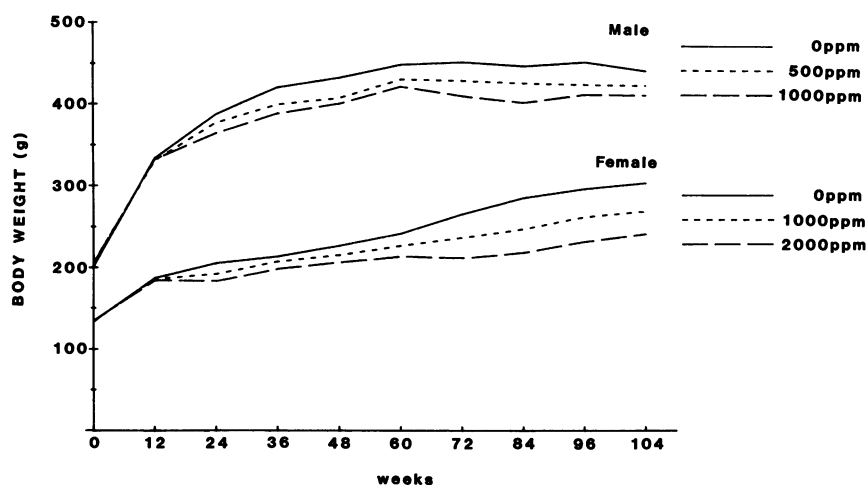
FIGURE 8.   Growth curves for male and female rats given NaClO.
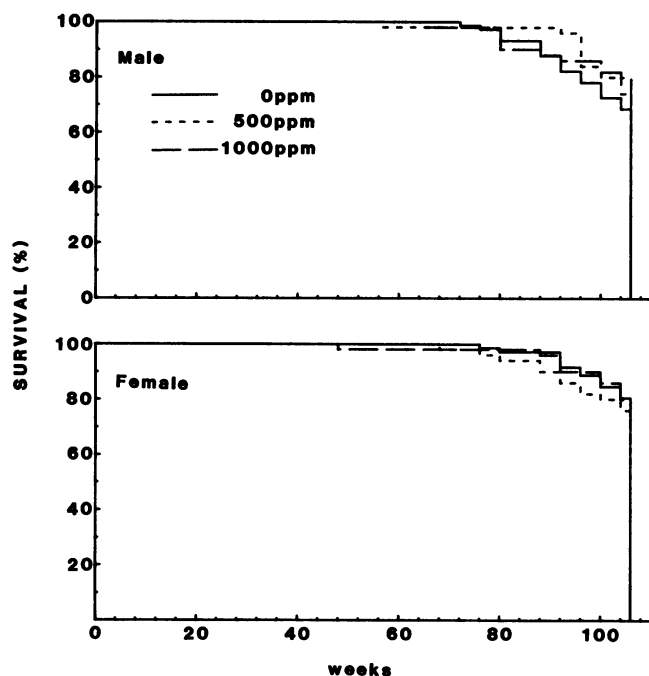
FIGURE 9. Survival curves for male and female mice given NaClO.

FIGURE 11. Survival curves for male and female rats given NaClO₂.

serum revealed significant decreases in glutamic-py-ruvic transaminase, the albumin-to-globulin ratio, serum potassium, and cholinesterase in females given 500 ppm KBrO₃. Also, slightly increased levels of blood urea nitrogen were observed in males and females treated with KBrO₃.

Mouse. Treatment of male mice was discontinued during this study (S. Takayama, unpublished data) because of severe fighting phenomena. Female mice were given KBrO₃ at doses of 1000 or 500 ppm for 78 weeks and thereafter given tap water for 26 weeks. All survivors were sacrificed at week 104. Although body weight increase was markedly inhibited in the high-dose group (Fig. 5), the survival curves were comparable, and sufficient numbers of animals survived for 104 weeks in all three groups (Fig.6). Daily intakes of KBrO₃ (mg/kg body weight/day) were 119.8 and 56.5 given at 1000 and 500 ppm, respectively.
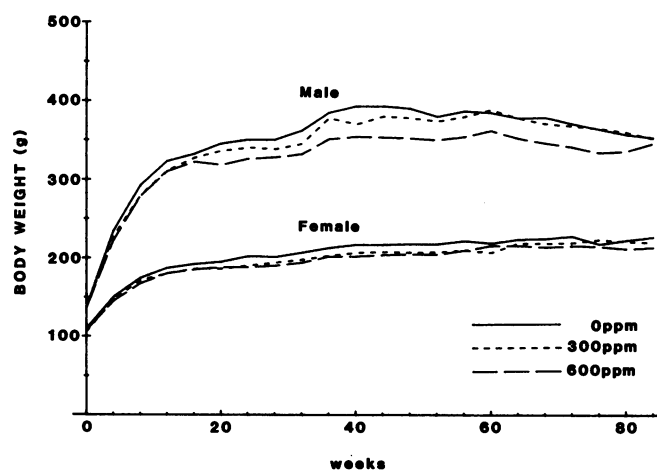
FIGURE 10. Growth curves for male and female mice given NaClO.

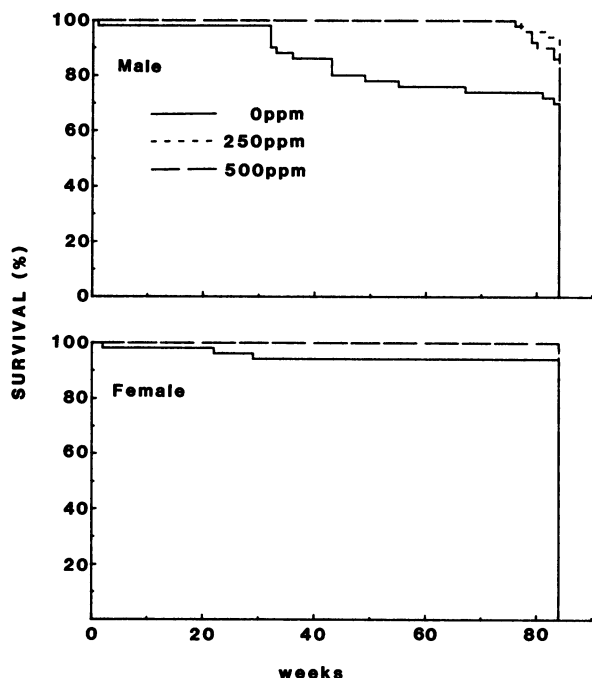FIGURE 12. Growth curves for male and female rats given NaClO₂.

FIGURE 13. Survival curves for male and female mice given NaClO₂.

Results of histologic examination of tumors are shown in Table 4. No significant differences in the incidences of tumor-bearing animals were apparent between treated and control groups. Although relatively high incidences of lung, liver and lymph node tumors were observed in the high-dose group, the incidences of these tumors were not significantly different from those of controls.

Carcinogenicity Tests on NaClO

Rat. In this test (M. Takahashi, unpublished data), NaClO was given orally at concentrations of 1000 or 500 ppm and 2000 or 1000 ppm, respectively, to males and females for 104 weeks. The rats were thereafter given distilled water, and all survivors were sacrificed at week 112. The survival ratios at week 104 were very high, ranging from 80% to 70%, in all groups of both sexes (Fig. 7). Dose-dependent inhibition of body weight increase was observed in both male and female rats (Fig. 8). Drinking water intakes were comparable among all groups.

All three groups demonstrated relatively high incidences of tumors of the testis, pituitary, thyroid, lung, pancreas, uterus, mammary gland, spleen, and subcutaneous tissue (Table 5). Histologically, chromophobic adenomas of the pituitary, adenomas and adenocarcinomas of C-cells of the thyroid, adenomas of the lung, insulomas of the pancreas, fibroadenomas of the mammary gland, and mononuclear cell leukemias were identified in both males and females. Also, high incidences of interstitial cell tumors of the testis and fibromas in subcutaneous tissue in males and endometrial polyps of

the uterus in females were observed. However, significant compound-related increases in tumor incidences were not found in any organs of treated animals of either sex. Incidences of nonneoplastic lesions, such as chronic nephropathy in treated males and granulomatous changes in the liver of treated females, were significantly decreased. Hematologic and serum biochemical analyses did not reveal significant dose-related changes in any parameters in either sex treated with NaClO.

Mouse. Groups of 50 male and 50 female mice were given NaClO at doses of 1000 or 500 ppm for 103 weeks (S. Asahina, unpublished data). However, control groups consisted or 73 males and 72 females in this test. All surviving mice were sacrificed at week 106, at which time the survival rates were 74%, 78%, and 66% in males and 78%, 80%, and 78% in females, respectively, for high-dose, low-dose, and control groups (Fig. 9). Dose-related reductions in body weight increase occurred in both sexes (Fig. 10). Total intakes (g/mouse/103 wks) of NaClO were 3.02 and 1.81 in males and 2.86 and 1.47 in females, respectively, in high- and low-dose groups.

Tumors occurring with relatively high incidences are listed in Table 6. Combined incidences of leukemias and malignant lymphomas and of adenomas and adenocarcinomas of the lung were very high in all groups for both sexes. Also, high incidences of hyperplastic nodules and hepatocellular carcinomas of the liver in males of all groups were noteworthy. However, statistically significant differences were not observed in tumor incidences for any organs of treated animals.

Carcinogenicity Tests on NaClO₂

Rat. This study (Y. Hiasa, unpublished data) was prematurely terminated at week 85 because of widespread Sendai viral infection in all groups, necessitating immediate sacrifice of all survivors. At necropsy, pneumonias were found in all animals, and an abscess of the lung had developed in some cases. Percentages of survivors at week 85 were 86%, 60%, and 68% in males and 100%, 88%, and 94% in females, respectively, in high-dose (600 ppm), low-dose (300 ppm), and control groups (Fig. 11). Body weight increase was inhibited in a dose-dependent manner in both males and females (Fig. 12). Drinking water intake in treated animals was slightly lower than that in control animals of both sexes. Daily consumption of NaClO₂ (mg/kg body weight/day) was 32.1 and 18.0 in males and 40.9 and 28.3 in females, respectively, for high- and low-dose groups.

No statistically significant differences in the incidence of tumor-bearing animals were observed between treatment and control groups of either sex. Incidences of tumors in several organs were appreciable (Table 7), i.e., C-cell adenomas of the thyroid, pheochromocytomas of the adrenal, and interstitial cell tumors of the testis in males, and chromophobic adenomas of the pituitary and endometrial polyps of the uterus in females. However, no statistically significant differences in the rates of tumor development in any organs were observed between NaClO₂-treated and control animals of

Table 5. Histopathologic diagnoses of tumors occurring at relatively high incidence in rats given NaClO.

	Male			Female		
	1000 ppm NaClO	500 ppm NaClO	0 ppm	2000 ppm NaClO	1000 ppm NaClO	0 ppm
Effective no. of rats	50	50	49	50	50	50
No. (%) of tumor-bearing rats	50 (100)	49 (98)	49 (98)	38 (76)	42 (84)	45 (90)
Pituitary	4 (8)	7 (14)	4 (8)	20 (40)	26 (52)	22 (44)
Adenoma (chromophobic)	4	7	4	20	26	21
Ganglioneuroma	—	—	—	—	—	1
Thyroid	7 (14)	11 (22)	7 (14)	3 (6)	8 (16)	3 (6)
Follicular adenoma	1	—	—	—	1	—
Follicular adenocarcinoma	—	1	—	—	—	—
Papillary adenoma	1	—	—	—	—	—
Papillary adenocarcinoma	1	—	—	—	1	—
C-cell adenoma	4	7	5	3	5	3
C-cell adenocarcinoma	—	3	2	—	1	—
Adrenal	3 (6)	2 (4)	10 (20)	1 (2)	4 (8)	—
Cortical adenoma	—	—	3	—	2	—
Pheochromocytoma	2	2	7	1	—	—
Malignant pheochromocytoma	1	—	—	—	2	—
Lung	10 (20)	4 (8)	6 (12)	1 (2)	2 (4)	4 (8)
Adenoma	6	4	6	1	2	3
Adenocarcinoma	3	—	—	—	—	—
Squamous cell carcinoma	1	—	—	—	—	—
Giant cell carcinoma	—	—	—	—	—	1
Pancreas	2 (4)	4 (8)	6 (12)	2 (4)	—	2 (4)
Insuloma	2	4	6	2	—	2
Liver	—	3 (6)	1 (2)	—	1 (2)	—
Adenoma	—	1	—	—	—	—
Hepatoma	—	—	1	—	1	—
Cholangioma	—	1	—	—	—	—
Malignant fibrous histiocytoma	—	1	—	—	—	—
Testis	49 (98)	48 (96)	49 (98)	—	—	—
Interstitial cell tumor	49	48	49	—	—	—
Uterus	—	—	—	14 (28)	12 (24)	9 (18)
Endometrial stromal polyp	—	—	—	6	7	5
Adenoma	—	—	—	1	—	2
Adenocarcinoma	—	—	—	6	3	2
Myoma	—	—	—	—	1	—
Fibroma	—	—	—	—	1	—
Malignant histiocytoma	—	—	—	1	—	—
Mammary gland	4 (8)	4 (8)	7 (14)	3 (6)	1 (2)	11 (22)
Adenoma	—	1	—	2	1	2
Fibroadenoma	4	2	6	1	—	8
Adenocarcinoma	—	1	1	—	—	1
Spleen	11 (22)	11 (22)	8 (16)	2 (4)	6 (12)	9 (18)
Leukemia	10	11	7	2	6	8
Hemangioma	—	—	—	—	—	1
Hemangioendothelioma	1	—	1	—	—	—
Subcutaneous tissues	7 (14)	4 (8)	10 (20)	2 (4)	—	—
Fibroma	6	3	9	2	—	—

either sex. Serum biochemistry analysis revealed that levels of glutamic oxaloacetic transaminase in the liver were significantly decreased in the high-dose males. Hematalysis and urinalysis revealed no significant changes in blood or urine.

Mouse. Mice in treatment groups of both sexes were given $NaClO_2$ at concentrations of 500 or 250 ppm for 85 weeks, at which time all survivors were sacrificed (Y. Konishi, unpublished data). Dead or moribund male mice were found during the experiment earlier in control groups than in the treated groups (Fig. 13) because of severe fighting. Survival percentages at the end of the study were 86%, 94%, and 70% in males and 100%, 100%, and 94% in females, respectively, in high-dose,

low-dose, and control groups. However, body weight increases were comparable among all groups of either sex (Fig. 14).
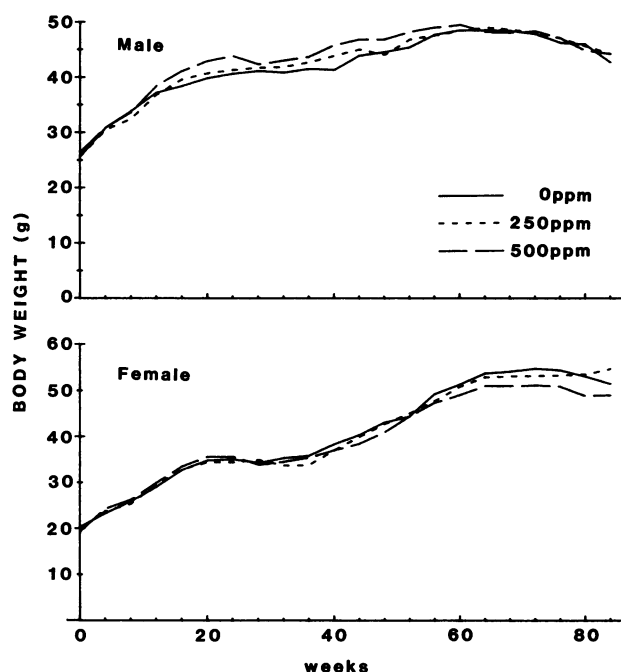
As shown in Table 8, the incidences of liver tumors were higher in treated males than in control males. These tumors were histologically diagnosed as hyperplastic nodules or hepatocellular carcinomas. The combined incidences of these tumors were significantly different in males of the low-dose group ($p < 0.05$). The incidences of hyperplastic nodules of the liver in males were significantly higher in both high- and low-dose groups ($p < 0.05$), although the incidences did not exhibit a dose-related effect. Also, the combined incidences of adenomas and adenocarcinomas and that of

Table 6. Histopathologic diagnoses of tumors occurring at relatively high incidence in mice given NaClO.

	Male			Female		
	1000 ppm NaClO	500 ppm NaClO	0 ppm	1000 ppm NaClO	500 ppm NaClO	0 ppm
Effective no. of rats	50	50	73	50	50	72
No. (%) of tumor-bearing mice	40 (80)	39 (78)	53 (73)	26 (54)	34 (68)	42 (58)
Pituitary	—	—	—	—	5 (10)	1 (1)
Adenoma	—	—	—	—	5	1
Hematopoietic organs	3 (6)	10 (20)	13 (18)	8 (16)	12 (24)	21 (29)
Leukemia/Malignant lymphoma	3	10	13	8	12	21
Spleen	3 (6)	—	3 (4)	2 (4)	2 (4)	—
Hemangioma	—	1	1	1	2	—
Hemangiosarcoma	3	—	2	1	—	—
Liver	24 (48)	27 (54)	31 (42)	3 (6)	4 (8)	9 (13)
Hyperplastic nodule	7	10	5	2	3	5
Hepatocellular carcinoma	15	17	24	1	1	4
Hemangioma	2	—	2	—	—	—
Lung	9 (18)	9 (18)	10 (14)	5 (10)	6 (12)	7 (10)
Adenoma/adenocarcinoma	9	9	10	5	6	7
Uterus	—	—	—	1 (2)	6 (12)	3 (4)
Endometrial stromal polyp	—	—	—	—	2	—
Adenocarcinoma	—	—	—	—	1	1
Endometrial stromal sarcoma	—	—	—	1	—	1
Leiomyoma	—	—	—	—	1	—
Leiomyosarcoma	—	—	—	—	—	1
Hemangioma	—	—	—	—	1	—
Hemangiosarcoma	—	—	—	—	1	—
Mammary gland	—	—	—	1 (2)	1 (2)	2 (3)
Fibroadenoma	—	—	—	1	—	1
Adenocarcinoma	—	—	—	1	—	1
Adenoacanthoma	—	—	—	—	—	1
Harderian gland	5 (10)	4 (8)	5 (14)	7 (14)	3 (6)	9 (13)
Adenoma	5	4	5	7	3	9
Skin and subcutis	2 (4)	2 (4)	4 (5)	1 (2)	1 (2)	2 (3)
Fibrosarcoma	—	—	1	—	—	—
Fibrous histiocytoma	1	—	—	—	—	—
Malignant fibrous histiocyt.	—	—	—	—	–	1
Angiofibroma	—	1	—	—	—	—
Hemangioma	1	—	1	—	—	—
Leiomyoma	—	—	1	—	—	—
Rhabdomyosarcoma	—	—	—	—	1	1
Malignant neurofibroma	—	1	1	—	—	—
Unclassified	—	—	—	1	—	—

Table 7. Histopathologic diagnoses of tumors occurring in rats given NaClO₂.

	Male			Female		
	600 ppm NaClO$_2$	300 ppm NaClO$_2$	0 ppm	600 ppm NaClO$_2$	300 ppm NaClO$_2$	0 ppm
Effective no. of rats	43	30	34	50	44	47
No. (%) of tumor-bearing rats	36 (83)	23 (76)	28 (82)	21 (42)	14 (31)	15 (31)
Pituitary	3 (7)	2 (7)	3 (9)	13 (26)	8 (18)	10 (21)
Adenoma	3	2	3	13	8	10
Thyroid	6 (14)	3 (10)	3 (9)	—	1 (2)	—
C-cell adenoma	6	3	3	—	1	—
Adrenal	7 (16)	3 (10)	9 (27)	3 (6)	—	—
Pheochromocytoma	7	3	9	3	—	—
Testis	29 (67)	22 (73)	24 (71)	—	—	—
Interstitial cell tumor	29	22	24	—	—	—
Uterus	—	—	—	7 (14)	5 (11)	6 (13)
Endometrial polyp	—	—	—	7	5	1
Adenocarcinoma	—	—	—	—	—	1
Mammary gland	1 (2)	—	—	—	—	—
Fibroma	1	—	—	—	—	—
Subcutis	1 (2)	—	—	—	—	—
Fibroma	1	—	—	—	—	—

FIGURE 14. Growth curves for male and female mice given NaClO₂.

adenomas of the lung were significantly higher in males of the high-dose group ($p < 0.05$). Relatively high tumor rates were observed for malignant lymphomas and/or leukemias and adenomas of the Harderian gland in both sexes, and for tumors of the liver and the lung in females of all groups. Of these, the incidences of malignant lym-

phomas and/or leukemias in the high-dose female group were smaller by a statistically significant margin.

Discussion

The design and conduct of the long-term assays described in this paper were considered adequate according to recent guidelines for carcinogenicity testing and evaluation of data (*1–3, 5, 6*), although the test on KBrO₃ in mice was carried out only in females, and that on NaClO₂ in rats of both sexes was stopped at week 85 because of infection. As a result, KBrO₃ was shown to have carcinogenic effects in rats of both sexes. NaClO₂ was negative in rats, although it generated equivocal data in male mice. However, no evidence of carcinogenicity was apparent for NaClO in both rats and mice of either sex.

KBrO₃ induced renal cell tumors in both male and female rats and mesotheliomas of the peritoneum in male rats when it was given orally at doses of 500 or 250 ppm for 110 weeks (*4,7*). The incidences of both tumors were significantly elevated and showed dose-response relationships. In the study of KBrO₃ using female mice, on the other hand, no statistically significant differences in the incidences of any tumors were observed between treatment and control goups. Kidney tumors found in this study were one adenocarcinoma and one adenoma in the high-dose group and one adenoma in the control group. Long-term oral administration of KBrO₃ to male mice of three strains (BDF1, CDF1 and B6C3F1) is currently underway at a dose of 750 ppm, based on the fact that kidney tumor incidence in mice is higher in males than in females (*8*).

Table 8. Histopathologic diagnosis of tumors occurring in mice given NaClO₂.

	Male			Female		
	500 ppm NaClO₂	250 ppm NaClO₂	0 ppm	500 ppm NaClO₂	250 ppm NaClO₂	0 ppm
Effective no. of rats	43	47	35	50	50	47
No. (%) of tumor-bearing mice	22 (51)	27 (57)	14 (40)	13 (26)	17 (34)	14 (30)
Liver	17 (40)	22 (47)*	7 (20)	6 (12)	5 (10)	5 (11)
Hyperplastic nodule	11 (26)*	14 (30)*	3	5	3	5
Hepatocellular carcinoma	6	8	4	1	1	—
Hemangioma	—	—	—	—	1	—
Lung	7 (16)*	3 (6)	—	2 (4)	2 (4)	3 (6)
Adenoma	5 (12)*	2	—	2	1	3
Adenocarcinoma	2	1	—	—	1	—
Malignant lymphoma/leukemia	1 (2)	2 (4)	4 (11)	1 (2)*	5 (10)	7 (15)
Harderian gland	1 (2)	3 (6)	1 (3)	4 (8)	4 (8)	2 (4)
Adenoma	1	3	1	4	4	2
Spleen	—	—	1 (3)	1 (2)	—	—
Hemangioma	—	—	1	1	—	—
Subcutis	2 (5)	2 (4)	—	—	—	—
Fibrosarcoma	1	2	—	—	—	—
Malignant fibrous histiocytoma	1	—	—	—	—	—
Pituitary	—	—	—	—	—	2 (4)
Adenoma	—	—	—	—	—	2
Thyroid	—	—	—	—	1 (2)	—
Follicular adenoma	—	—	—	—	1	—
Ovary	—	—	—	—	1 (2)	—
Papillary adenoma	—	—	—	—	1	—

*$p < 0.05$ (Fisher's exact probability test).

Table 9. Dose-response studies for renal lesion development after oral administration of KBrO₃ for 104 weeks in male F-344 rats.

Dose of KBrO₃, ppm	Effective no. of rats	Dysplastic foci (DF)			Renal cell tumors (RCT)		
		Incidence of DF (%)	Total no. of DF	No. of DF per rat ±SD	Incidence of RCT (%)	Total no. of RCT	No. of RCT per rat
500	20	19(95)[a]	146	7.30 ± 5.60	9(45)*	25	1.25
250	20	19(95)[a]	84	4.20 ± 2.40	5(25)†	5	0.25
125	24	12(50)[a]	19	0.79 ± 1.04	5(21)†	5	0.21
60	24	6(25)[b]	8	0.33 ± 0.62	1(4)	1	0.04
30	20	5(25)[b]	5	0.25 ± 0.43	0	0	0
15	19	1(5)	1	0.05 ± 0.22	0	0	0
0	19	0	0	0	0	0	0

*p < 0.001.
†p < 0.05 (Fisher's exact probability test).

Table 10. Results of mutagenicity tests on KBrO₃, NaClO, and NaClO₂.[a]

Test	KBrO₃	NaClO	NaClO₂
Ames test	Positive	Positive	Positive
DNA repair test	Negative	Positive	Not tested
Chromosome aberration test	Positive	Positive	Positive
Micronucleus test	Positive	Negative	Positive

[a] Data taken from references (30–32) or supplied as personal communication from Dr. M. Ishidate, Department of Mutagenesis, National Institute of Hygienic Sciences.

Long-term feeding studies for KBrO₃ in Great Britain have been reported (9, 10). A basal bread diet prepared from untreated flour or from flour treated with 50 or 75 ppm of KBrO₃ was given to rats (9) and mice (10) for 104 and 80 weeks, respectively. No evidence of carcinogenicity or chronic toxicity was observed in either experiment. The discrepancy in results can be explained by the finding that almost all KBrO₃ added to flour at normal food additive levels is converted to potassium bromide (KBr) during the normal British baking process, and the amount of KBrO₃ is negligible in the final products (11,12). Therefore, commercial bread supplemented with KBrO₃ was found to be noncarcinogenic to rats and mice in the British studies. In contrast, our test demonstrated positive potential carcinogenicity of KBrO₃, which itself is very stable in water, in rats of both sexes when administered in their drinking water.

Recently, long-term dose-response studies on this chemical have been completed (Y. Kurokawa, unpublished data). Male F344 rats were given KBrO₃ orally for 104 weeks at concentrations of 500, 250, 125, 60, 30, or 15 ppm (Table 9). Statistically significant increases were observed in the incidences of renal cell tumors in rats treated with > 125 ppm. In addition, incidences of dysplastic foci, considered to be preneoplastic lesions of renal cell tumors (13,14), were significantly higher in rats treated with > 30 ppm. The virtually safe dose (VSD) of KBrO₃ for renal cell tumors was estimated by the probit model with independent background (S. Aoki, unpublished data). The VSD at risk levels of 10^{-6} and 10^{-8} was 0.950 and 0.302 ppm, respectively (p = 0.877).

To determine further species differences in the carcinogenicity of KBrO₃, male Syrian golden hamsters were treated orally for 89 weeks (Y. Kurokawa, unpublished data). In this study, no statistically significant differences were observed in the incidences of tumors in any organs. However, the rates of renal cell tumors were 11% (2/19), 20% (4/20), 6% (1/17), 0% (0/19), and 0% (0/20), respectively, in groups given 2000, 500, 250, 125, or 0 ppm. Although the incidences were relatively low and were not statistically different, the results seemed to indicate the potential for KBrO₃ to induce renal cell tumors in male hamsters, inasmuch as the incidences of spontaneous kidney tumors were extremely low in this rodent species (15-17).

The promoting effect of KBrO₃ was also investigated. KBrO₃ given orally at a concentration of 500 ppm was found to have a promoting effect on renal, but not on liver, tumorigenesis, when initiated with N-ethyl-N-hydroxyethylnitrosamine (EHEN) in male F344 rats (14). Subsequently, dose-response studies in two-stage renal carcinogenesis were undertaken to ascertain whether a threshold level of KBrO₃ treatment exists for its promoting activity (18). Male F344 rats were treated orally at doses of 500, 250, 125, 60, 30, or 15 ppm for 24 weeks after initiation with EHEN. The results showed that the mean numbers of kidney dysplastic foci were significantly increased in a dose-related manner when KBrO₃ was given at doses of > 30 ppm. Therefore, the threshold level for enhancing renal tumorigenesis seemed to lie between 30 and 15 ppm. The potential enhancing effect of KBr was also tested in this study, with the result that it was considered negative for renal carcinogenesis (18).

From studies on carcinogenicity and on promoting effects, we concluded that KBrO₃ possessed both initiating and promoting activities, the characteristics of a complete carcinogen, for development of renal cell tumors in rats. In contrast, KBrO₃ exhibited neither promoting nor complete carcinogenic activities for skin carcinogenesis (19).

Several experiments were conducted to elucidate the mechanism of carcinogenic action of KBrO₃ (Y. Kurokawa, unpublished data). In acute toxicity studies, KBrO₃ induced a dose-dependent increase in lipid peroxidation in the kidney. Survival times after a single intravenous administration of KBrO₃ were extended by

pretreatment with glutathione in male F344 rats. Ultrastructurally, extensive damage to the mitochondria of the renal tubular epithelium was observed. The appearance of multiple eosinophilic bodies in the cytoplasm of proximal renal tubules in $KBrO_3$-treated rats was noteworthy as a nonneoplastic change in the kidney. These droplets were strongly stained with eosin and were positive with the Azan stain, but were negative for the periodic-acid Schiff stain (20).

In the test of $NaClO_2$, no evidence of carcinogenicity was apparent in rats of either sex. On the other hand, marginal increases were observed in the incidences of liver and lung tumors in male mice given $NaClO_2$. Namely, statistically significant increases were found in the combined incidences of hyperplastic nodules (HN) and hepatocellular carcinomas (HCC) of the liver in a low-dose group, and adenomas and adenocarcinomas of the lung in a high-dose group in males (both $p \leq 0.05$). It should be mentioned, however, that males in the control group died spontaneously, earlier than treated males in this study because of severe fighting (Fig. 13) and were not included in the effective numbers for histopathologic examination. On the other hand, respective incidences of HN and HCC of the liver were 27.8% (range, 22–33%) and 20.1% (range, 8–23%) in 194 untreated B6C3F1 males in earlier bioassays in our program (S. Fukushima, unpublished data). Also, the incidences of adenomas and adenocarcinomas of the lung were 9.8% (range, 6–16%) and 5.7% (range, 2–8%), respectively, in the same controls. Note that, although statistically elevated over those of concurrent controls, the rates of HN of the liver in the high-dose (25.6%) and low-dose (29.8%) groups and that of lung adenomas in the high-dose group (11.6%) were all within the range of historical control data for our program as well as that of the National Toxicology Program (21). We concluded, threrefore, especially since significant increases were observed only in the incidences of benign tumors, that the results of the carcinogenicity test on $NaClO_2$ in mice were inconclusive under the conditions of this study.

Several toxicological studies have reported results of tests on chlorine and chlorite because of the unavoidable human exposure to these substances in drinking water after disinfection (22–26). The potential toxicity of hypochlorous acid (HOCl) when orally administered to male Sprague-Dawley rats for 12 months at concentrations of 100, 10, or 1 ppm was investigated (26). Significant increases were observed in glutathione levels and osmotic fragility of the erythrocytes. Red blood cell count and hematocrit were significantly decreased. Also, incorporation of $[^3H]$thymidine into kidney and testis nuclei was increased. The results of long-term oral treatment with highly chlorinated water containing 100 ppm of free chlorine was reported (27). BD11 rats were given chlorine over their life span in seven consecutive generations. No compound-related toxicity or carcinogenicity was observed. $NaClO_2$ was given to male Sprague-Dawley rats in their drinking water for 12 months at levels of 100 or 10 ppm (25). Various hematologic parameters were affected in the treatment group similarly to HOCl-treated rats (26). However, such hematologic changes were not observed in rats and mice of both sexes given NaClO or $NaClO_2$ in our carcinogenicity tests and in a subchronic study (28). Although positive promoting activity has been reported for the ddN-strain mouse skin with application of a 10% solution of NaClO (29), this effect was not found when a 1% NaClO solution was applied to SENCAR mouse skin (19). $NaClO_2$ painted at a concentration of 100 ppm on SENCAR mouse skin was suspected of exerting skin promoting activity (19).

Results of mutagenicity testing of the three compounds are summarized in Table 10 (30–32); all three gave positive results in three out of the four mutagenicity tests conducted. Therefore, these compounds could be classified as highly mutagenic compounds. Nonetheless, neither NaClO nor $NaClO_2$ appeared carcinogenic in our studies, although the carcinogenicity of $KBrO_3$ was clearly demonstrated.

$KBrO_3$, NaClO, and $NaClO_2$ have been used for a variety of purposes using their oxidizing properties, a characteristic common to all three compounds. With respect to the in vivo carcinogenic and promoting actions of oxidant chemicals, recent reports on mouse skin carcinogenesis deserve mention.

Benzoyl peroxide was shown to be a potent promoter (33) and a possible complete carcinogen (19) in this system and was suspected as a causative agent for skin cancer in humans (34). Hydrogen peroxide, lauroyl peroxide, decanoyl peroxide, cumene peroxide (35, 36), NaClO (29), and $NaClO_2$ (19) were demonstrated to be relatively weak as promoters, whereas $KBrO_3$ and ammonium persulfate were inactive either as complete carcinogens or as promoters in mouse skin carcinogenesis (19). In systems other than the skin, hydrogen peroxide given orally was found to have carcinogenic potential in mice by inducing duodenal tumors (37,38) and to have promoting action by increasing intestinal tumor development in rats (39). Although $KBrO_3$ showed a promoting effect in two stage rat renal carcinogenesis (14,18), no such influence was evident for potassium chlorate and sodium chlorate given orally at a level of 1% for 24 weeks after initiation with EHEN (Y. Kurokawa, unpublished data).

It seems generally accepted that carcinogenic and promoting action of these compounds both in vivo and in vitro are caused by various kinds of active oxygen species, e.g., superoxide, singlet oxygen, hydroxy radical, and hydrogen peroxide (40–42). From extensive toxicological studies on oxidant chemicals such as paraquat (43), ozone (44), and NO_x (45), it was revealed that these chemicals could induce lipid peroxidation in their target organs. Induction of lipid peroxidation and clastogenic actions are now considered to be the underlying mechanisms of carcinogenic and/or promoting effects shown by the oxidant chemicals (40, 46). Indeed $KBrO_3$ increased the levels of lipid peroxide in the kid-

ney (Y. Kurokawa, unpublished data) and was clasto-
genic in Chinese hamster cells (31). In spite of the ac-
cumulating evidence of the role of free oxygen radicals
in in vitro promotion and cell transformation, the num-
ber of in vivo studies available to support in vitro data
remains insufficient (47). Although the skin seems to be
a suitable target organ for detecting potential carcino-
genic and promoting effects of oxidants (36), the effects
of these compounds on gastrointestinal organs should
also be tested in view of their local action as skin irri-
tants. The fact that humans are inevitably exposed or-
ally to food additives and to by-products of water dis-
infection having oxidizing characters also emphasizes
the need to test these chemicals for carcinogenic and
promoting effects in organs other than skin.

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